# IL-6 levels after exposure to lipopolysaccharide through injection or alcohol consumption

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University of Texas at Austin
Dean's Scholars Honors Thesis
2008

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**Abstract**

Alcohol interacts with the immune system in multiple ways that are not fully understood but which likely contribute to alcoholic pathologies and may be relevant to the study and treatment of alcohol dependence. Alcohol consumption permeabilizes the gut, allowing the release of lipopolysaccharide (LPS), an endotoxin that elicits the production of pro-inflammatory cytokines (protein signaling molecules produced by immune cells) including interleukin-6 (IL-6). As excessive inflammation may lead to shock, there exists a protective mechanism, endotoxin tolerance, that suppresses the inflammatory response to LPS after an initial exposure. Because of the phenomenon of endotoxin tolerance, we hypothesized that chronic alcohol exposure would result in the suppression of the IL-6 response to LPS.

In this study, mice were treated with a single injection of LPS, repeated injections of LPS, or one injection of LPS after a period of chronic alcohol dinking, and then the levels of IL-6 were measured in order to assess the immune response. It was found that while mice given a single injection of LPS showed a significant increase in IL-6, those given repeated injections of LPS did not show any increase; they had developed tolerance to the multiple exposures of LPS. After 18 days of chronic voluntary intake of alcohol, the IL-6 response to LPS was also suppressed. This shows that chronic alcohol consumption produces an alteration in the immune system that could play a possible role in the development of alcohol dependence. Further research is necessary to determine if this area of study could lead to the better understanding and treatment of alcohol dependence.

## Background

The human gastrointestinal tract is home to 500-1000 bacterial species (Sears), including Gram-negative species such as *E. coli*. Gram-negative bacteria, which are characterized by certain cell wall structures, contain in their outer membranes a structural component called lipopolysaccharide (LPS), a molecule composed of lipid A, a core oligosaccharide, and an O polysaccharide side chain (Lu et al.). The mammalian body recognizes LPS, an endotoxin, as a sign of bacterial infection and therefore responds with a potent immune response- inflammation. LPS stimulates TLR4 receptors, a class of pattern recognition receptors called toll-like receptors, found on the surfaces of macrophages, monocytes, and other immune cells of the innate immune system, activating a pathway that leads to the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6)

Fig. 1 The human gut contains an abundant bacterial flora. The inset shows a scanning electron micrograph of part of the small intestine, with bacteria shown in green. (Bajzer and Seeley)

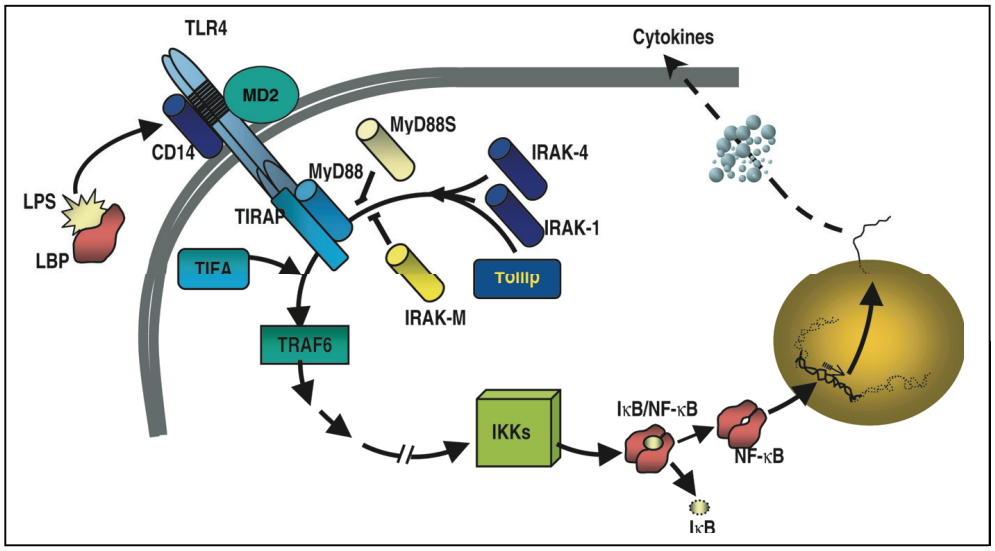


Fig. 2 LPS activates the TLR4 signal transduction pathway, leading to the production of pro-inflammatory cytokines. (Villar et al.)

(Medvedev et al.). These protein signaling molecules, produced in response to infection or cell damage (Romeo et al.), are mediators of the inflammation response and produce inflammation in multiple tissues, including the liver and the brain (Crews et al.).

It is not clear exactly how the LPS produced by bacteria in the gut does not induce an inflammatory response within the gut, but it has been suggested that cells displaying TLRs are compartmentalized within the submucosa and therefore are physically separated from the bacterial flora (Sears). However, if LPS is translocated from the gastrointestinal tract into circulation as a result of damage to or increased permeability of the lining of the gut, an immune response is triggered (Asai et al.).

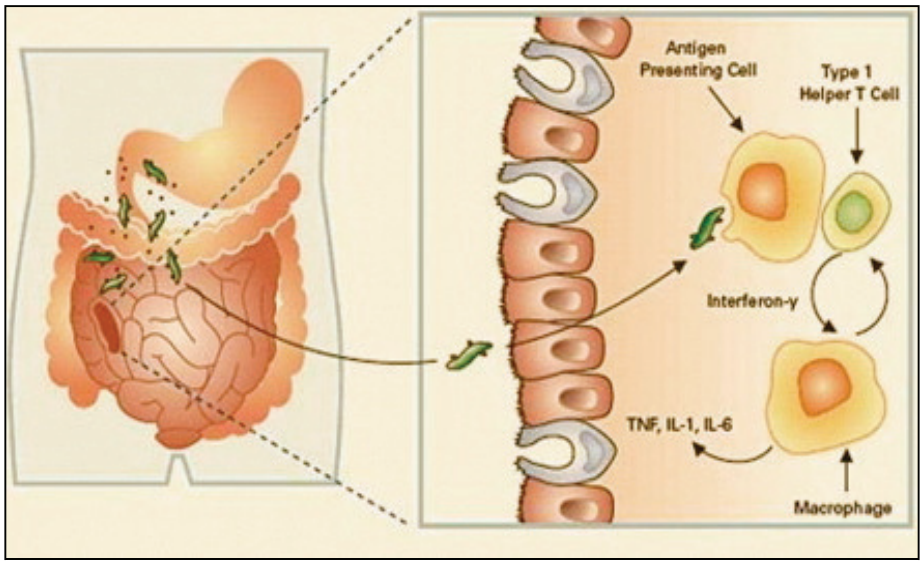


Fig. 3 LPS released from the gut elicits an immune response. (image courtesy of www.redlabs.be)

Alcohol has been shown to increase gastrointestinal permeability, creating a "leaky gut" that allows the release of LPS (Tang et al.). In rats, gut permeability was seen to increase by 3-fold after chronic ethanol treatment, with an accompanying increase in portal endotoxin levels (Enomoto et al. 2001). The mechanisms by which alcohol changes barrier function are not completely understood. While high concentrations of ethanol (35%-50%) cause macroscopic injury to the intestinal epithelium (the cells composing the lining of the intestine) that obviously and readily allows the translocation of

LPS, even low concentrations of ethanol (7.5%) have been shown to produce changes in tight junction proteins and epithelial permeability (Asai et al.).

Tight junctions, the close connections between adjacent epithelial cells, are composed of proteins and regulate intestinal permeability. Zonula occludens 1 (ZO-1) is a major protein of tight junctions and is known to be modulated by alcohol. Ethanol induces an increase in the expression of miR-212, a microRNA (miRNA) that targets ZO-1 (Tang et al.). MiRNAs are single-stranded RNA molecules that regulate gene expression by complementarily binding to the messenger RNAs (mRNAs) of their target genes, thereby inhibiting protein synthesis. The ethanol-induced increase in MiR-212 consequently results in decreased expression of ZO-1, disrupting the integrity of intestinal tight junctions and resulting in barrier hyperpermeability.

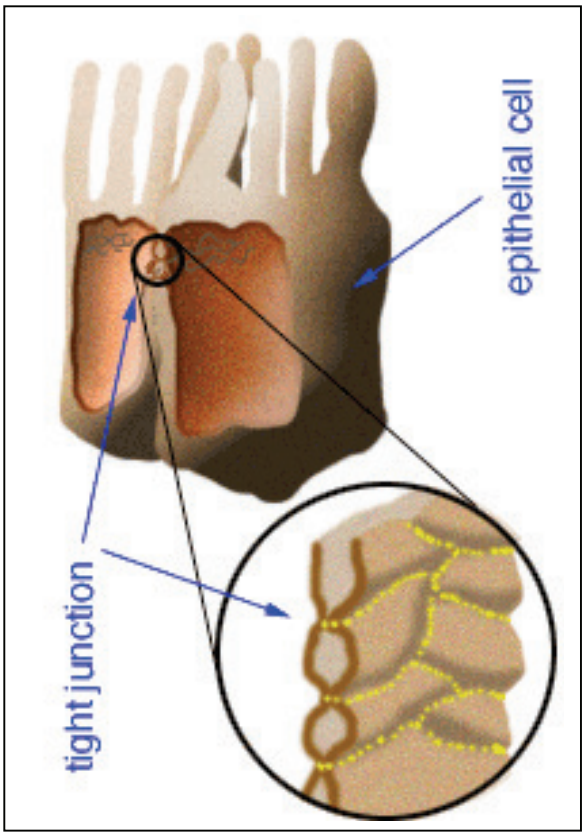


Fig. 4 Tight junctions regulate the passage of materials between epithelial cells. Alcohol disrupts tight junctions, allowing LPS to exit the gut. (image courtesy of John Blamire)

Low concentrations of alcohol induce apoptosis, or programmed cell death, of intestinal cells (Asai et al.). Apoptosis causes cell shrinkage, producing gaps between adjacent cells that lead to increased permeability (Asai et al.). These alcohol-induced epithelial cell changes, and perhaps others, likely account for the increased gastrointestinal permeability found in some alcoholics (Bode and Bode), especially those with alcoholic liver disease (Keshavarzian, et al.).

Endotoxemia (the presence of endotoxins in the blood) resulting from increased gut permeability has been noted in alcoholics (Yamashina et al.). Furthermore, alcoholics are known